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MOLECULAR MODELS

Readings: Review pp. 22-29, 35-39, and 47-54 in your text (POHS, 5th ed.).

Introduction
The concepts of molecular structure and function are crucial to understanding the laboratory exercises in Biology 201. Textbook drawings of molecules are limited because they are two-dimensional. While the drawings (also called *projections*) act as a useful shorthand, they fail to reveal the ways in which molecules interact with each other and with themselves.

Model building is a good way to begin to grasp how bond geometry determines the larger geometry of entire molecules. As you construct models of glucose and alanine, keep in mind which parts of each molecule will come into contact with or bond to other molecules.

LAB GOALS
This exercise is designed to help you practice the art of three-dimensional imagination by building models of biologically important molecules.

EXPERIMENTAL PROTOCOL
Molecular model building. Work individually.

A. Description of model components.
You will be using color-coded jacks and straws to build molecular models. The color code of the straws is as follows:

red	=	oxygen
black	=	carbon
white	=	hydrogen
blue	=	nitrogen

Each straw represents a covalent bond between the atoms indicated by the color(s) of the straw; a white-red straw represents a hydrogen-oxygen bond, black-black is a carbon-carbon bond, etc. Two kinds of jacks are available:

gold $= sp^2$ -hybridized carbon atom; bond angles are 120°, shape is triangular.
silver $= sp^3$ -hybridized carbon atom; bond angles are 109°, shape is tetrahedral.

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B. Constructing an amino acid.
Using the chemical formula and appropriate jacks and straws, construct the backbone of alanine, one of the 20 amino acids found in proteins. Depending on the orientation of the R group added to the central carbon, you will have constructed either D- or L-alanine. D- and L- forms are non-superimposable mirror images of each other. The *perspective* drawings below illustrate this feature. Bonds indicated by dotted lines extend behind the plane of the paper, bonds shown by wedges extend toward you from the plane of the paper, and bonds represented by solid lines are in the plane of the paper.
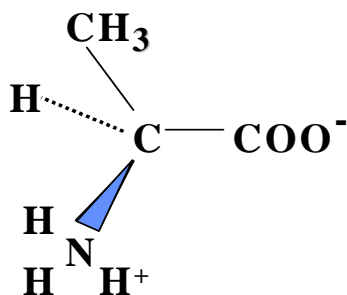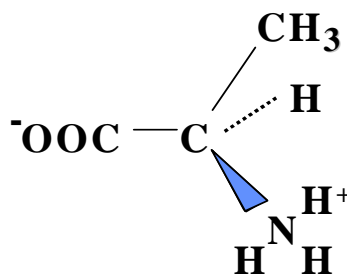

**L-Alanine**   **D-Alanine**

Note that the central carbon in alanine is **chiral**; that is, the central carbon is asymmetric and is bonded to four different groups. (With the exception of glycine, all amino acids occur in D- and L- forms.) **Only L-amino acid residues occur naturally in proteins.**

C. Constructing a sugar
Using the formula in the figure below and the appropriate jacks and straws, construct a model of D-glucose. (As with the amino acids, only one D- or L- form of monosaccharide occurs naturally in biological molecules. **In the case of sugars, it is the D-form.**) The drawing on the left in the following figure is a *Fischer projection*. In Fischer projections, the molecule is arranged so that horizontal bonds project above the plane of the paper and vertical bonds project behind it. This is a method of portraying molecules so that the arrangement of their components may be easily seen, and is generally not a good predictor of how the molecule will look in three dimensions. Your model will be curved, and can be converted into a ring by joining the carbonyl carbon (carbon$_1$) to the oxygen attached to carbon$_5$. This reaction occurs when glucose is in solution with water, also shown in the following figure.
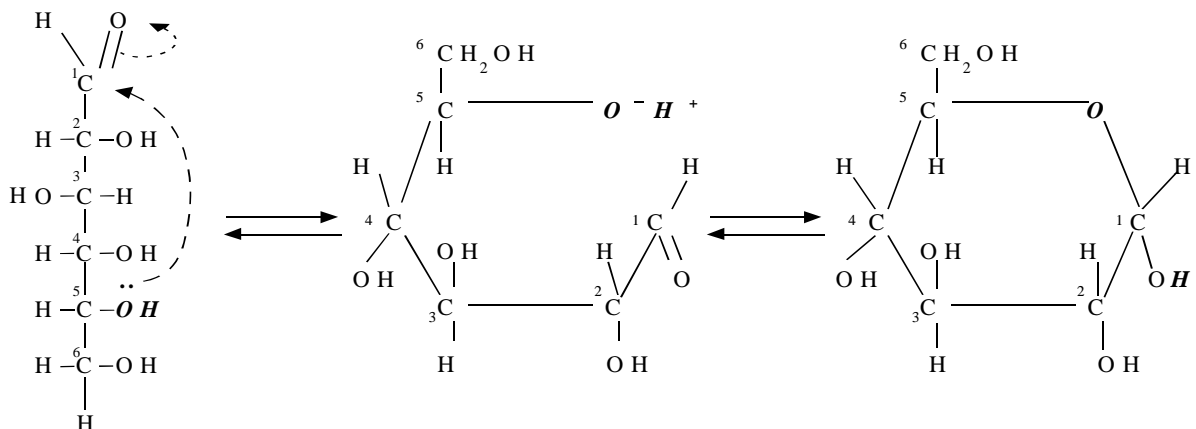
The configuration of the molecule is determined by the direction of the C_1 hydroxyl relative to the plane of the ring. If the hydroxyl group points upward, the molecule is in the β-configuration; a downward pointing hydroxyl group yields an α-configuration (see illustration below).

α Configuration   β Configuration
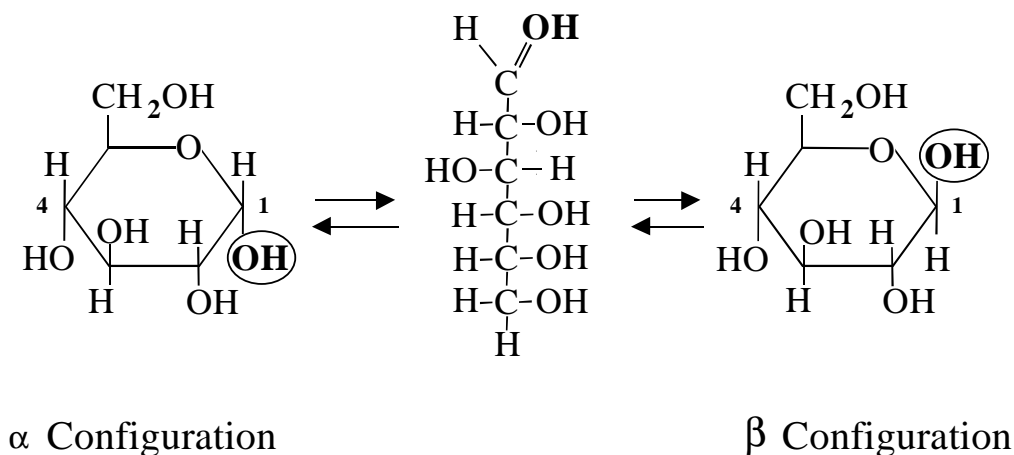
Find three or more other students who have constructed D-glucose in the same configuration you have. Join your molecules together (i.e., make a polysaccharide). Polymerized β-D-glucose forms cellulose, while polymerized α-D-glucose forms starch. If you make starch, make sure you can see why the molecule is curved. If you make cellulose, understand why the molecule is straight.

DATA ANALYSIS and LAB WRITE UP
No formal write up is required for this exercise.

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TECHNIQUES and CALCULATIONS

Readings: None.

LAB GOALS
(1) Learn to pipet accurately, and **(2)** brush up on mathematical manipulations used in subsequent exercises.

EXPERIMENTAL PROTOCOLS
1. Pipetting and Serial Dilution. Work in pairs.

Many laboratory manipulations require making defined, less-concentrated (or diluted) solutions from stock solutions. Pipets allow accurate transfer of small volumes of liquids. [Note: pipets are very expensive; please handle them carefully. If the pipet tip is broken or chipped, the pipet can no longer be used accurately.] Blue and green pipet-aids are available for your use (mouth pipetting is absolutely forbidden). The blue pipettor is used with the 1.0 ml pipet **only**, and the green pipettor is used with 5.0 or 10.0 ml pipets. Examine the pipets and become familiar with the subdivisions on each size. Insert the non-tip end of the pipet into the pipet-aid firmly but gently and twist the pipet so you can read the markings on it. Hold both the pipet and pipettor in one hand so you can rotate the wheel to either take up or expel liquid. Practice pipetting known volumes of water until you feel confident of your technique. Set up a rack with tubes arranged as shown in the following sketch. Label your tubes with small pieces of tape. Make sure you **remove all tape** before putting your tubes into the wash tub.
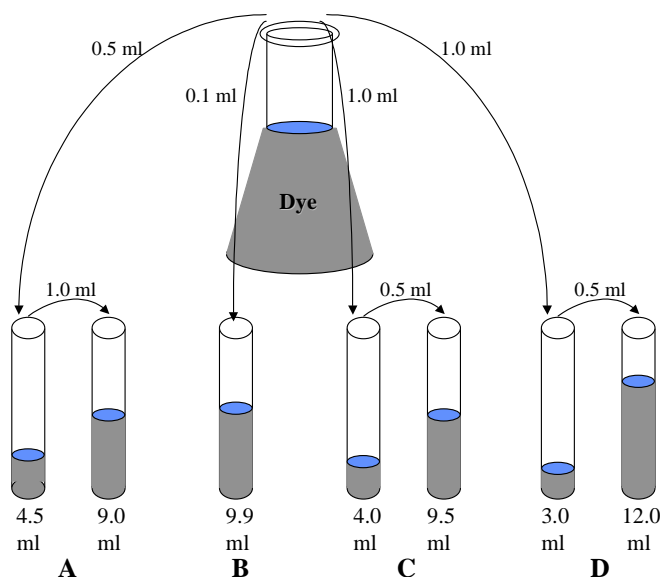
Use one 10.0 ml pipet to deliver the amounts of water indicated below the tubes to each tube; these tubes are your water blanks. Use one 1.0 ml pipet to deliver the amounts of dye indicated above the arrows to the first water blank in each dilution series. Mix your tubes by flicking the bottoms gently. Use separate 1.0 ml pipets to complete each dilution series. Hold the most dilute (least concentrated) tubes from each of the serial dilutions against a piece of white paper and compare the colors visually.

Use equation (*a*) to calculate how dilute the solution is in each tube (the individual dilution factor):

$$\text{(a) Individual dilution factor} = \frac{\text{volume added}}{\text{volume added} + \text{volume in blank}}$$

Use equation (*b*) to calculate how dilute the solution in the final tube in the series is in comparison to the concentration in the original sample (the total dilution factor):

(*b*) Total dilution factor = (individual dilution factor)(individual dilution factor)(etc.)

Pipetmen are devices used to transfer very small volumes. A P100 can accurately deliver volumes between 10 µl and 100 µl, and a P1000 is used to deliver volumes between 100 µl and 1000 µl. Your instructor will tell you how to use the pipetmen. Add 10 µl dye to 990 µl H_2O in another tube. Compare this tube visually with the final tubes in each of the series.

● Calculate the total dilution factor for dilution series A, B, C, D, and compare it to the dilution factor in the dilution you made with the pipetmen.

● Design a serial dilution scheme that would give a total dilution factor of 1.0×10^5.

2. Calculations used in Biology 201. Work individually.
Complete the mathematics section on your own. See your instructor during office hours for individual help, if needed. These calculations should be done without the aid of a calculator.

Most reagents in biology use the metric system. The table below shows the relationships of some standard metric units. <u>Memorize these relationships.</u>

Volume:

milliliter	ml	10^{-3} liter
microliter	μl	10^{-6} liter
nanoliter	nl	10^{-9} liter

Concentration:

millimolar	mM	10^{-3} moles/liter
micromolar	μM	10^{-6} moles/liter
nanomolar	nM	10^{-9} moles/liter

Mass:

milligram	mg	10^{-3} gram
microgram	μg	10^{-6} gram
nanogram	ng	10^{-9} gram

Length:

millimeter	mm	10^{-3} meter
micrometer	μm	10^{-6} meter
nanometer	nm	10^{-9} meter

Solutions:

Molar solutions are based on how many molecules of a substance there are in a given solution. In order to make molar solutions, you must know the molecular or formula weight of a substance – this can almost always be found on the container. The molecular weight of NaCl, for instance, is 58.5 g/mole.

Example: To prepare 1 liter of a 1 M solution of NaCl, 58.5 g of NaCl would be dissolved in a total volume of 1 liter H_2O.

● How would you make 500 ml of 1 M NaCl?
● How would you make 300 ml of 2.5 M NaCl?
● How would you make 100 ml of 50 mM NaCl?

Mass/volume solutions are based on the concentration of a chemical in a given solution. *Example*: 10 mg/ml Bovine Serum Albumin is 10 mg of Bovine Serum Albumin dissolved in a total volume of 1 ml.

Percentage solutions are a form of mass/volume solution expressed in terms of percent. *Example*: 10% NaCl would be made by dissolving 10 g NaCl in a total volume of 100 ml.

Dilutions:

Making solutions correctly is a crucial skill in the laboratory. A concentrated "stock" solution is frequently prepared; this "stock" is then used to prepare less concentrated (or diluted) solutions for experimental use. The dilution factor is the relationship between the concentration of a stock solution and the concentration of the final solution; that is,

Dilution factor $=$ stock solution concentration / final concentration

The following is a general formula for using dilution factors to calculate the volume of stock solution to add when making a solution for use:

Final volume of solution / dilution factor $=$ volume of stock solution used

Example: To make 250 ml of 10 mM NaCl from a 500 mM NaCl stock –
(a) 500 mM stock / 10mM final $=$ 50 (dilution factor)
(b) 250 ml (final volume) / 50 (dilution factor) $=$ 5.0 ml of 500 mM NaCl stock
(c) Add 5.0 ml 500 mM NaCl to 245 ml H_2O to make 250 ml 10 mM NaCl

● Describe how to make 500 ml of 50 µM NaCl from a stock solution of 500 mM NaCl.
● Describe how to make 100 ml of 5 mM NaCl, 1mM EDTA from stock solutions of 200 mM NaCl and 100 mM EDTA.
● Describe how to make 500 ml of 25 mM NaCl, 10 mM EDTA, 1.5 mM $MgCl_2$ from the following stock solutions: 500 mM NaCl, 500 mM EDTA, 15 mM $MgCl_2$. (Don't forget to include H_2O to reach the final volume.)

DATA ANALYSIS and WRITE-UP
No formal write up is required for this exercise. Make certain you can perform the calculations indicated in Parts 1 and 2!